JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1988, p. 1910–1912
0095-1137/88/091910-03$02.00/0
Copyright © 1988, American Society for Microbiology
Vol. 26, No. 9

New Selective Medium for *Pseudomonas aeruginosa* with Phenanthroline and 9-Chloro-9-[4-(Diethylamino)Phenyl]-9,10-Dihydro-10-Phenylacridine Hydrochloride (C-390)

MAUREEN E. CAMPBELL,[1,2] SUSAN W. FARMER,[1,2] AND DAVID P. SPEERT[1,2,3]*

Departments of Pediatrics[1] and Microbiology,[3] University of British Columbia, and Division of Infectious Diseases, British Columbia's Children's Hospital,[2] Vancouver, British Columbia, Canada

Received 25 April 1988/Accepted 13 June 1988

A new selective medium (PC agar) for the isolation of *Pseudomonas aeruginosa* was developed, consisting of 30 μg of 9-chloro-9-[4-(diethylamino)phenyl]-9,10-dihydro-10-phenylacridine hydrochloride (C-390) per ml and 30 μg of phenanthroline per ml in Columbia agar. PC agar was superior to phenanthroline, C-390, acetamide, and cetrimide agars for the selective growth of *P. aeruginosa*.

Pseudomonas aeruginosa is an important opportunistic pathogen in patients with neutropenia, thermal burns, and cystic fibrosis. It owes its pathogenicity in part to its intrinsic resistance to antibiotics (7). Neutropenic patients are often infected with strains of *P. aeruginosa* after colonization of the lower gastrointestinal tract (2). Other patients, such as those with thermal burns, nosocomial pneumonia, or cystic fibrosis (11), might also infect themselves with their endogenous strains of *P. aeruginosa*.

To better understand this process of autoinfection and to design rational strategies for its prevention (5, 6), a sensitive means of detecting *P. aeruginosa* in low numbers from mixed cultures (such as the feces) is required. Several selective media for *P. aeruginosa* have been described (1, 3, 8–10, 12–14, 16), but none is absolutely specific. We describe a new selective agar medium containing phenanthroline and 9-chloro-9-[4-(diethylamino)phenyl]-9,10-dihydro-10-phenylacridine hydrochloride (C-390) which has superior sensitivity and specificity for *P. aeruginosa*.

A total of 335 gram-positive and 1,978 gram-negative strains were studied (see Table 2). Bacteria were kindly provided by John Anderson from clinical specimens submitted to the British Columbia's Children's Hospital diagnostic microbiology laboratory; by Allison Clarke from Metropolitan Laboratory, Vancouver; by John Smith from Vancouver General Hospital; and from the research collections of David Scheifele and Marie Gribble. All bacteria were identified by conventional diagnostic microbiological techniques.

Cetrimide agar (Difco Laboratories, Detroit, Mich.) and Columbia agar (Oxoid Ltd., Basingstoke, England) were prepared by the manufacturers' recommendations. Acetamide agar contained 20.0 g of acetamide (BDH Chemicals, Toronto, Ontario, Canada), 5.0 g of NaCl, 1.0 g of $NH_4H_2PO_4$, 1.0 g of K_2HPO_4, and 15.0 g of Bacto-Agar (Difco) in 1 liter of distilled water. Phenanthroline agar contained 30 mg of phenanthroline (BDH) and 39 g of Columbia agar per liter of distilled water. C-390 medium contained 30 mg of C-390 (Norwich Eaton Pharmaceuticals, Norwich, N.Y.) and 39 g of Columbia agar per liter of distilled water. PC medium contained 30 mg of phenanthroline, 30 mg of C-390, and 39 g of Columbia agar per liter of distilled water.

Both broth and agar dilution MICs were determined with phenanthroline and C-390 (Table 1). Broth MICs were measured in acetamide broth as the sole carbon source (9) with phenanthroline (0, 0.5, 1, 2, 4, 8, 16, 31, 62, 125, 250, 500, and 1,000 μg/ml) or with C-390 (0 and 1.5 to 100 μg/ml in serial twofold steps). Bacteria were grown overnight on Columbia agar containing 5% sheep blood, inoculated at 10^5/ml into each broth tube, and incubated at 37°C with vigorous agitation for 18 h. The MIC was interpreted as the lowest concentration of the added chemical at which turbidity was not visible. Agar dilution MICs were determined with phenanthroline (0, 2, 3, 4, 5, 10, 20, 30, 40, 50, 75, and 100 μg/ml) or with C-390 (0, 10, 15, 17.5, 20, 25, 30, 40, 50, 75, and 100 μg/ml) in Columbia agar. Bacteria were grown for 18 h on Columbia agar containing 5% sheep blood, suspended in saline, and adjusted to a McFarland turbidity standard of 0.5. A 3-μl aliquot of each strain was inoculated onto each of the agar plates by using a replicator (Cathra International, Inc., Minneapolis, Minn.), and the plates were incubated at 37°C overnight. The MIC was interpreted as the lowest concentration of the inhibitor at which there was no visible growth.

There were substantial differences in MIC results between the agar and broth methods. *P. aeruginosa* was the only species tested with high-level resistance to both substances in the agar dilution method. All strains were therefore tested for growth on agar with phenanthroline and/or C-390 added at 30 μg/ml. All 2,313 bacterial strains (335 gram positive and 1,978 gram negative) were prepared as for the agar dilution MIC determination and inoculated by replicator to Columbia, acetamide, cetrimide, phenanthroline, C-390, and PC agar media. The plates were incubated for 18 h at 37°C, and each strain was assessed for visible growth (Table 2). Of the 1,456 strains of *P. aeruginosa*, all grew on phenanthroline, 1,452 grew on both C-390 and PC agar, 1,408 grew on acetamide, and 1,433 grew on cetrimide. C-390 and PC agar were superior to the other media in selectivity for *P. aeruginosa*, and PC was clearly the best. Only *P. aeruginosa* grew on PC agar, whereas 7 strains of *Achromobacter* spp., 5 strains of *Aeromonas hydrophila*, 11 strains of *Klebsiella* spp., 8 strains of *Enterobacter* spp., and 1 strain of *Serratia* sp. grew on C-390. Phenanthroline (30 μg/ml) was not selective for *P. aeruginosa*; it supported the growth of both gram-positive and gram-negative strains. Nonetheless, when phenanthroline was added to C-390 (PC medium) the few non-*P. aeruginosa* strains that grew on C-390 were sup-

* Corresponding author.

TABLE 1. MICs of phenanthroline and C-390 for different gram-negative bacteria

Bacterial species	MIC (μg/ml)			
	Agar dilution		Broth dilution	
	Phenanthroline	C-390	Phenanthroline	C-390
Achromobacter xylosoxidans	30	>100	8	6
Acinetobacter calcoaceticus	30	10	8	25
Serratia marcescens	>100	10	8	3
Providencia stuartii	>100	10	8	3
Proteus mirabilis	>100	10	8	3
Escherichia coli				
Lactose fermenting	>100	10	4	1.5
Non-lactose fermenting	>100	10	4	1.5
Pseudomonas aeruginosa[a]				
Classic	>100	>100	62	100
Mucoid	>100	>100	31	100
Dwarf	>100	>100	125	>100

[a] Colony morphology of *P. aeruginosa* strains is described in reference 15.

pressed or did not grow but there was no further suppression of *P. aeruginosa*. Cetrimide agar inhibited the growth of all gram-positive strains, and acetamide agar inhibited the growth of most gram-positive strains. Growth on the five media was unaffected after storage of the bacteria at −70°C.

Recovery of *P. aeruginosa* from sites where it is present in low numbers or where other microorganisms are also present provides a considerable challenge. Strategies to enhance recovery of *Pseudomonas* spp. have included the use of selective media (1, 8, 12, 16) and enrichment by passage through acetamide broth (9, 14). Although both of these approaches have been found to improve the isolation rate of *P. aeruginosa*, neither is perfect. Selective media are not absolutely specific for *P. aeruginosa*; they may inhibit *Pseudomonas* spp. (4) as well as permit the growth of other antibiotic-resistant gram-negative bacilli (1, 8, 10).

Selective media for isolation of *P. aeruginosa* include nutrient agar supplemented with antibiotics (12, 16), cetrimide agar (4, 10), *Pseudomonas* isolation agar (with irgasan) (4), and growth media supplemented with C-390 (1, 3, 8, 13) or with phenanthroline (J. E. Keeven and B. T. DeCicco, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C355, p. 382). Of all these selective media, those supplemented with C-390 have been the most sensitive and specific for the recovery of *P. aeruginosa* (13). Nonetheless, C-390 is not a perfect selective agent; other gram-negative bacteria are capable of growing in its presence (1). Furthermore, it is potentially capable of inhibiting the growth of *P. aeruginosa* (4). To obviate this potential problem, we combined low concentrations of C-390 with phenanthroline, an agent which suppresses the growth of some other gram-negative bacteria but supports the normal growth of *P. aeruginosa*.

This new selective medium (PC agar), which consisted of Columbia agar supplemented with C-390 and phenanthroline, was superior to either of these selective agents used alone at the same concentration and was far more sensitive and specific than either acetamide or cetrimide agar. Phenanthroline supported the growth of all strains of *P. aeruginosa* tested but also permitted many other gram-positive and gram-negative strains to grow. C-390 agar was more specific for *P. aeruginosa*, but several *Achromobacter*, *Aeromonas*, *Klebsiella*, *Enterobacter*, and *Serratia* strains were able to grow. PC agar was as sensitive as C-390 for the recovery of *P. aeruginosa*, but it was 100% specific; no other gram-negative bacteria were recovered. The mechanism by which addition of phenanthroline enhanced the specificity of the C-390 medium is not known. We suspect that the two agents

TABLE 2. Growth of bacterial strains on different selective agar media

Bacterial species	No. tested	No. of strains grown on agar:					
		Columbia	Acetamide	Cetrimide	Phenanthroline	C-390	PC
Pseudomonas aeruginosa	1,456	1,456	1,408	1,433	1,456	1,452	1,452
Pseudomonas maltophilia	75	75	73	1	36	0	0
Pseudomonas cepacia	17	17	14	9	8	0	0
Pseudomonas fluorescens	11	11	10	8	11	0	0
Pseudomonas putida	6	6	4	4	4	0	0
Other *Pseudomonas* spp.	9	9	8	3	1	0	0
Achromobacter spp.	13	13	13	13	13	7	0
Aeromonas hydrophila	28	28	28	4	2	5	0
Other nonfermenting gram-negative bacilli	26	26	26	2	4	0	0
Escherichia coli	139	139	106	1	14	0	0
Proteus spp.	20	20	6	14	7	0	0
Morganella sp.	1	1	0	1	0	0	0
Providencia spp.	6	6	4	6	5	0	0
Citrobacter spp.	19	19	18	1	17	0	0
Klebsiella spp.	96	96	88	9	73	11	0
Enterobacter spp.	47	47	40	15	29	8	0
Serratia spp.	9	9	9	8	1	1	0
Staphylococcus aureus	137	137	0	0	135	0	0
Coagulase-negative staphylococci	126	126	0	0	122	0	0
Streptococcus spp.	61	61	1	0	57	0	0
Diphtheroids	10	10	3	0	0	0	0
Bacillus spp.	1	1	0	0	1	0	0

were additive or synergistic against those strains able to grow in the presence of either one alone.

PC agar could prove to be practical for enhancing recovery of *P. aeruginosa* from sources containing other bacterial species, such as stool, waste water, and environmental samples. It may also improve detection of *Pseudomonas* spp. from sites where the bacteria are present in very small numbers, such as the throats of patients with cystic fibrosis prior to the development of frank respiratory infection. This new medium may also prove to be useful for presumptive identification of *P. aeruginosa* in diagnostic laboratories in which inoculation of different media is used to characterize gram-negative bacilli.

This work was supported by grants from the Canadian Cystic Fibrosis Foundation, the British Columbia Health Care Research Foundation, and the National Health Research and Development Program. D. P. Speert is a scholar of the Canadian Cystic Fibrosis Foundation.

We thank David Scheifele, John Anderson, and Nevio Cimolai for their critical reviews of the manuscript.

LITERATURE CITED

1. **Araj, G. F.** 1984. Use of 9-chloro-9-(4-diethylaminophenyl)-10-phenylacridan as a primary medium for recovery of *Pseudomonas aeruginosa* from clinical specimens. J. Clin. Microbiol. **20:**330–333.
2. **Cohen, M. L., M. T. Murphy, G. W. Counts, C. D. Buckner, R. A. Clift, and J. D. Meyers.** 1983. Prediction by surveillance cultures of bacteremia among neutropenic patients treated in a protective environment. J. Infect. Dis. **147:**789–793.
3. **Davis, J. R., C. E. Stager, and G. F. Araj.** 1983. 4-h identification of *Pseudomonas aeruginosa* with 9-chloro-9-(4-diethylaminophenyl)-10-phenylacridan. J. Clin. Microbiol. **17:**1054–1056.
4. **Fonseca, K., J. MacDougall, and T. L. Pitt.** 1986. Inhibition of *Pseudomonas aeruginosa* from cystic fibrosis by selective media. J. Clin. Pathol. **39:**220–222.
5. **Guiot, H. F. L., J. W. M. van der Meer, and R. van Furth.** 1981. Selective antimicrobial modulation of human microbial flora: infection prevention in patients with decreased host defense mechanisms by selective elimination of potentially pathogenic bacteria. J. Infect. Dis. **143:**644–654.
6. **Guiot, H. F. L., and R. van Furth.** 1984. Selective antimicrobial modulation of the intestinal flora. Prophylaxis against infection in neutropenic patients. Infection **12:**1–4.
7. **Hancock, R. E. W.** 1985. The *Pseudomonas aeruginosa* outer membrane permeability barrier and how to overcome it. Antibiot. Chemother. **36:**95–102.
8. **Havelaar, A. H., and M. During.** 1986. C-390 as sole selective agent for isolation of *Pseudomonas aeruginosa* from hospital waste water. Can. J. Microbiol. **32:**513–515.
9. **Kelly, N. M., F. R. Falkiner, and C. T. Keane.** 1983. Acetamide broth for isolation of *Pseudomonas aeruginosa* from patients with cystic fibrosis. J. Clin. Microbiol. **17:**159.
10. **Lambe, D. W., Jr., and P. Stewart.** 1972. Evaluation of Pseudosel agar as an aid in the identification of *Pseudomonas aeruginosa*. Appl. Microbiol. **23:**377–381.
11. **Lonnqvist, B.** 1986. Is resistance to colonization in the digestive tract relevant for bronchial infection in cystic fibrosis? J. Infect. Dis. **153:**175–176.
12. **Maduri-Traczewski, M., C. L'Heureux, L. Escalona, A. Macone, and D. Goldmann.** 1986. Facilitated detection of antibiotic-resistant *Pseudomonas* in cystic fibrosis sputum using homogenized specimens and antibiotic-containing media. Diagn. Microbiol. Infect. Dis. **5:**299–305.
13. **Marold, L. M., R. Freedman, R. E. Chamberlain, and J. J. Miyashiro.** 1981. New selective agent for isolation of *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. **41:**977–980.
14. **Smith, R. F., and S. L. Dayton.** 1972. Use of acetamide broth in the isolation of *Pseudomonas aeruginosa* from rectal swabs. Appl. Microbiol. **24:**143–145.
15. **Wahba, A. H., and J. H. Darrell.** 1965. The identification of atypical strains of *Pseudomonas aeruginosa*. J. Gen. Microbiol. **38:**329–342.
16. **Wong, K., M. C. Roberts, L. Owens, M. Fife, and A. L. Smith.** 1983. Selective media for the quantitation of bacteria in cystic fibrosis sputum. J. Med. Microbiol. **17:**113–119.